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# A Thermodynamic Study of the Association of the Acid Form of Methyl Orange with Cyclodextrins

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## *ABSTRACT*

*The UV-visible spectrum of the first conjugate acid of methyl orange was investigated at pH of 1·1 and at several temperatures in the presence of α-, β- and γ-cyclodextrins in aqueous solutions. The presence of γ-cyclodextrin has no measurable effect on the spectrum, while α- and β-cyclodextrins induced relatively larger changes in the absorbances of the visible region. These changes were analyzed according to a 1:1 inclusion process and the equilibrium constants were determined at four temperatures. The values of the thermodynamic quantities* $\Delta H^\circ$, $\Delta S^\circ$ *and* $\Delta G^\circ$ *were obtained for the inclusion processes of α- and β-cyclodextrins. The inclusion complex of α-cyclodextrin is more stable than that of β-cyclodextrin. The findings were rationalized on the basis of variations in the size of a cyclodextrin cavity.*

## 1 INTRODUCTION

A variety of azo dyes have been studied as guest molecules that could be accommodated in the cavity of a cyclodextrin in aqueous solutions. Hersey and Robinson[1] studied the thermodynamics and kinetics of the association of α-cyclodextrin with several azo-salicylates and reported formation constants of 1:1 inclusion complexes that ranged from $1{\cdot}3 \times 10^3$ to $9{\cdot}9 \times 10^3\ \mathrm{mol^{-1}\,dm^3}$ at 25°C. The forward and reverse rate constants for the inclusion processes of such azo-salicylates were found to be very dependent on the nature of the substituent groups on the phenyl ring. Rohrbach and Wojcik[2] have investigated the steric and charge effects on the association of

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α-cyclodextrin with several azobenzene dyes. According to Yoshida *et al.*,[3] azo dyes containing a naphthalene moiety form less stable inclusion complexes compared with those containing no naphthalene moiety. It has been reported[3] that the azo dyes containing the naphthalene moiety penetrate the α-cyclodextrin cavity via the aromatic ring that contains the sulphonate group. In view of the kinetic specificity noted in their work, Cramer *et al.*[4] have concluded that dye association at the outer side of the α-cyclodextrin cavity should be ruled out; instead, the inner side of the cavity ought to be the binding site. In support of this idea, azo dyes having two naphthalene moieties, on both sides of the azo linkage, were reported not to bind with α-cyclodextrin.[5] Owing to their relatively larger cavities, β- and γ-cyclodextrins have the tendency to bind larger azo dyes that usually exhibit no inclusion with α-cyclodextrin. The formation of ternary inclusion complexes with β- and γ-cyclodextrins is very likely where the azo dye is incorporated as a dimer in the cavity of the cyclodextrin.[6,7]

Methyl orange (mostly in the alkaline form) has been extensively studied in connection with its inclusion complexes with α-, β- and γ-cyclodextrins. The effect of these cyclodextrins on the visible spectrum of methyl orange was exploited in studying the inclusion processes of other substrates which compete with methyl orange for binding with these cyclodextrins such as alcohols[8] and 4-substituted phenols.[9] It has been proposed[1] that the acidic and basic forms of methyl orange bind to the α-cyclodextrin cavity via the *N*,*N*-dimethylaniline side, resulting in the formation of 1:1 inclusion complexes. Other equilibrium studies have also reported on the inclusion complexes of methyl orange with α-, β- and γ-cyclodextrins.[10-13]

In a previous publication from the author's laboratory, the tautomerism of the acid form of methyl orange was taken into consideration when dealing with the inclusion process with α- and β-cyclodextrins.[12] However, the lack of literature information on the tautomeric constant and molar absorptivities of model compounds at temperatures other than 25°C has limited the study to one temperature.

The present study is concerned with reporting the thermodynamic parameters $\Delta H^\circ$, $\Delta S^\circ$ and $\Delta G^\circ$ which represent the apparent standard values of changes in the enthalpy, the entropy and the Gibbs energy of the inclusion processes of the acid form of methyl orange with α- and β-cyclodextrins.

## 2 EXPERIMENTAL

The sodium salt of methyl orange ($C_{14}H_{14}N_3SO_3Na$) was purchased from Sigma (St Louis, MO, USA). Before its use, a test sample was dried at 90°C in an oven for about 16 h. A typical stock solution of methyl orange had a concentration of $1{\cdot}86 \times 10^{-4}$ mol dm$^{-3}$ and a pH of 10. The samples of α-, β- and γ-cyclodextrins (α-CD, β-CD and γ-CD) were also obtained from Sigma. The cyclodextrins were weighed as stable hydrates. These hydrates were found to have $6H_2O$ molecules for α-CD, $9H_2O$ molecules for β-CD, and $8H_2O$ molecules for γ-CD. The concentrations reported in this work were adjusted to the dry basis. Stock solutions of about $6 \times 10^{-3}$ mol dm$^{-3}$ were prepared by weighing the required amount of the hydrated cyclodextrin followed by dissolving in distilled, deionized water. The solutions needed for studying the formation of the inclusion complexes were prepared by transferring a fixed amount (about 5 ml) of the methyl orange stock solution to a 50 ml volumetric flask (25 ml volumetric flasks were used for γ-CD solutions), followed by adding the required amount of a cyclodextrin stock solution, then diluting to the mark with aqueous HCl. The pH of such solutions was adjusted to 1·1. The concentration of a cyclodextrin was varied in the range $1 \times 10^{-4}$ to $4{\cdot}6 \times 10^{-3}$ mol dm$^{-3}$. Reagent grade NaCl was used to vary the ionic strength up to 0·26 mol dm$^{-3}$. The volume measurements were carried out by using a 10 ml microburette. The UV-visible spectra of the test solutions were recorded at four temperatures in the range 14–40°C for α- and β-cyclodextrins and 5–40°C for γ-cyclodextrin. A double-beam spectrophotometer (DMS 100, Varian) and stoppered quartz cells with optical path length of 1·00 cm were used. A constant temperature water bath was used to keep the cells' block at the required temperature within ±0·02°C.

## 3 RESULTS AND DISCUSSION

The effect of both α- and β-cyclodextrins on the UV-visible spectrum of the acid form of methyl orange was found to be similar to that reported previously.[12] Both cyclodextrins induced an appreciable decrease in the intensity of the visible absorption and an increase in the intensity of the UV absorption. Figure 1 illustrates the effect of α-cyclodextrin on the absorbance of the acid form of methyl orange at its maximum absorption ($\lambda = 505$ nm) where the absorbance decreases with increase of the concentration of α-cyclodextrin. The same behaviour was also observed at 525, 530 and 535 nm under different experimental conditions of temperature, ionic strength, and the concentration of methyl orange. Similar observations were also obtained in the case of β-cyclodextrin. However, under similar experimental conditions, it is found that α-cyclodextrin causes larger changes in absorbance compared with β-cyclodextrin. For example, $9{\cdot}24 \times 10^{-4}$ mol dm$^{-3}$ α-cyclodextrin decreases the absorbance at 525 nm by 38%, while the same amount of β-cyclodextrin decreases the absorbance by 9% at 25°C.

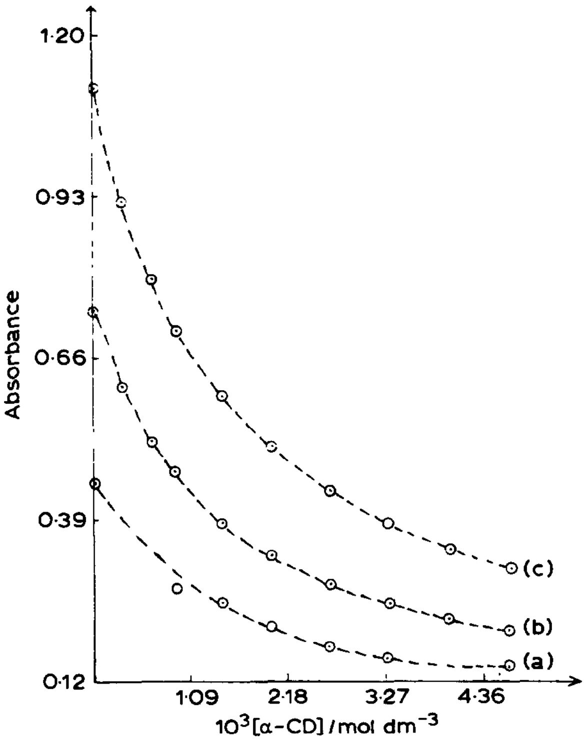

Fig. 1. The effect of the concentration of $\alpha$-cyclodextrin ($\alpha$-CD) on the absorbance of the acid form of methyl orange at a wavelength of 505 nm, temperature of 25°C and ionic strength of 0·10 mol cm$^{-3}$. The initial concentrations of methyl orange are $1{\cdot}114 \times 10^{-5}$, $1{\cdot}857 \times 10^{-5}$ and $2{\cdot}786 \times 10^{-5}$ mol dm$^{-3}$ for curves a, b and c, respectively. The circles represent experimental data and the dotted curves are interpolations.

The alterations in the spectrum, as described above, were interpreted as a result of the association of the acid form of methyl orange with $\alpha$- and $\beta$-cyclodextrins. In principle, more than one stoichiometry can be proposed for the inclusion process. In addition, the *cis-trans* isomerism and the tautomerism of the first conjugate acid of methyl orange[14] requires the consideration of several inclusion complexes for a given stoichiometry. The investigation of all these possibilities was not feasible in this work. Instead an overall inclusion process was considered and the spectrophotometric data were analyzed on the basis of the following 1:1 inclusion process:

$$\mathrm{CD} + \mathrm{MO} \overset{K}{\rightleftharpoons} \mathrm{CD.MO} \tag{1}$$

where the symbols CD, MO, CD. MO and $K$ represent a cyclodextrin, the acid form of methyl orange, the inclusion complex and the equilibrium constant of the inclusion process, respectively.

In order to obtain $K$ from the absorbance measurements, the Benesi–Hildebrand equation[15] was applied in the following form:

$$l.C_0S_0/\Delta A = (1/K.\Delta\varepsilon) + C_0/\Delta\varepsilon \qquad (2)$$

where $l$ is the optical path length of the cell used (1·00 cm). $C_0$ and $S_0$ represent the initial concentrations (in mol dm$^{-3}$) of a cyclodextrin and the acid form of methyl orange, respectively. $\Delta A$ is the change in the absorbance of methyl orange due to the addition of a cyclodextrin, and $\Delta\varepsilon$ is the difference in the molar absorptivities between the free and complexed methyl orange. Equation (2) is a straight line equation with slope equal to $1/\Delta\varepsilon$ and intercept equal to $1/K.\Delta\varepsilon$. The equilibrium constant, $K$, is given by the slope/intercept ratio. It is assumed in this study that the equilibrium given by eqn (1) represents an apparent inclusion process that arises from the participation of more than one isomeric and/or tautomeric form of the acid form of methyl orange.

The absorbance data obtained at several wavelengths were treated according to eqn (2). Figures 2 and 3 show the plot of eqn (2) at 505, 525 and 535 nm for the α- and β-cyclodextrin inclusion processes at 25°C. The data of

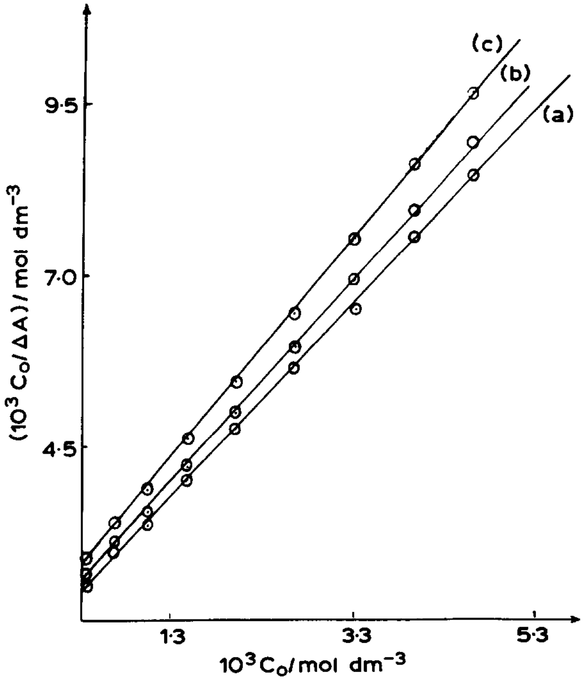

Fig. 2. The plot of eqn (2) for α-cyclodextrin/methyl orange system at 25°C and ionic strength of 0·20 mol dm$^{-3}$. The circles represent experimental data while the lines a, b and c are the least squares fit at wavelengths of 505, 525 and 535 nm, respectively.

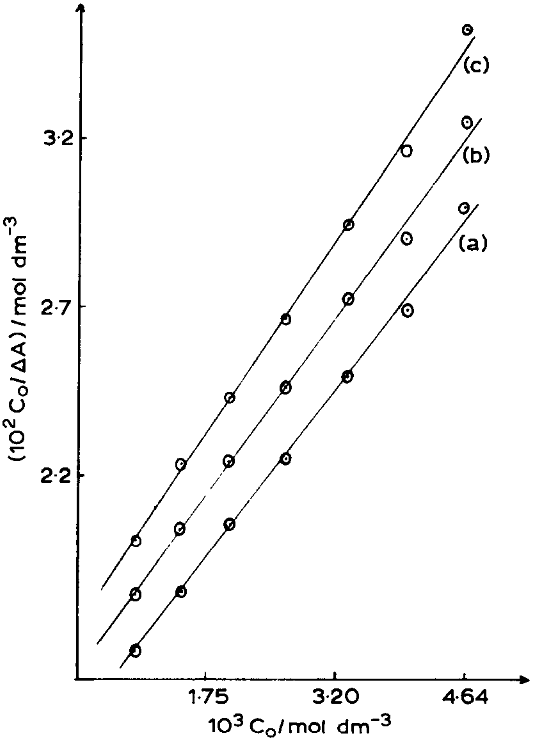

**Fig. 3.** The plot of eqn (2) for β-cyclodextrin/methyl orange system at 25°C and ionic strength of 0·26 mol dm$^{-3}$. The circles represent experimental data while the lines a, b and c are the least squares fit at wavelengths of 505, 525 and 535 nm, respectively.

Figs 2 and 3 were in accord with the linearity suggested by eqn (2), with correlation coefficients of 0·99 or better. It is found that, for a given data set (six to nine solutions in a set), the value of $K$ at a certain wavelength was within $\pm 1\%$ from the mean of the set, implying that $K$ is wavelength independent. However, the evaluation of $K$ from data in the UV region (at $\lambda_{max} = 316$ nm) was not considered because of the small changes in absorbances. The equation given by Hirai *et al.*[10] shows that the slope/intercept ratio depends on the initial concentration of an azo dye for a 2:1 (MO/CD) inclusion process. However, the ratio is independent of the initial concentration of the azo dye for a 1:1 inclusion process as given by eqn (2). Three sets of data (of the type shown in Fig. 1) having different values of the initial concentration of methyl orange, $S_0$, were analyzed according to eqn (2) and the slope/intercept ratio of each set was within $\pm 3\%$ of the mean of the three sets for both α- and β-cyclodextrins. These findings support the proposal of the 1:1 stoichiometry as given in eqn (1).

The effect of temperature on the inclusion process given by eqn (1) was examined at four temperatures in the range 14–40°C for α- and β-cyclodextrins. It is found that, as the temperature is increased, the effect of both α- and β-cyclodextrins on the absorbances of methyl orange decreases. Figure 4 shows plots of eqn (2) for the α-cyclodextrin/methyl orange system at 505 nm at four temperatures. Other data collected at 525, 530 and 535 nm were subjected to a linear least squares analysis and an average value for $K$ was obtained at each temperature. The same procedure was followed for obtaining $K$ values for the β-cyclodextrin/methyl orange system at four temperatures.

In order to evaluate the standard enthalpy change, $\Delta H^\circ$, and the standard entropy change, $\Delta S^\circ$, of the inclusion process given by eqn (1), the values of $K$ obtained at four temperatures were analyzed according to eqn (3)

$$\log K = -(\Delta H^\circ/2{\cdot}303R)/T + (1/2{\cdot}303R)\,\Delta S^\circ \qquad (3)$$

It should be pointed out that the equilibrium constant, $K$, given in eqn (3) is a thermodynamic one, while $K$ in eqn (2) is an apparent equilibrium constant defined in terms of concentrations and might be affected by changes in the

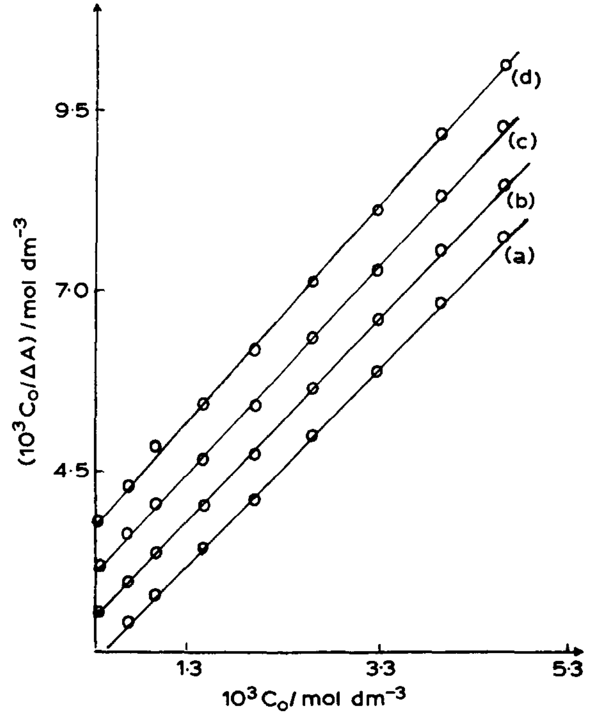

Fig. 4. The plot of eqn (2) for α-cyclodextrin/methyl orange system at an ionic strength of 0·20 mol dm$^{-3}$ and wavelength of 505 nm. The circles represent experimental data while the lines a, b, c and d are the least squares fit at 14·6, 25·0, 32·8 and 39·9°C, respectively.

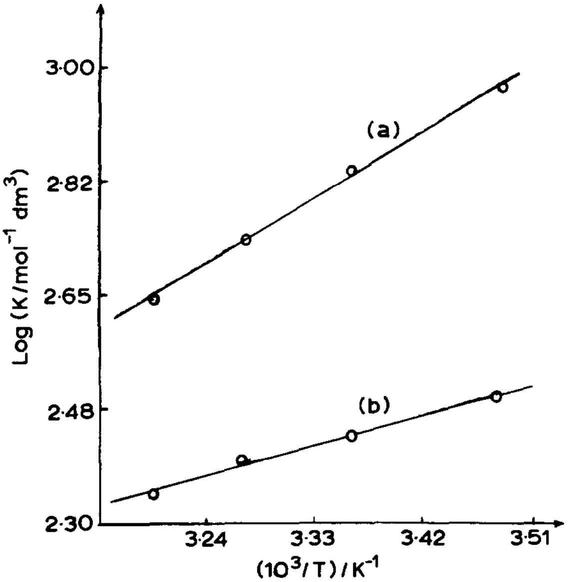

**Fig. 5.** The plot of log $K$ versus $1/T$. The circles represent experimental data while the lines a and b are the least squares fit for the data of α-cyclodextrin and β-cyclodextrin systems, respectively.

ionic strength of the solution. It was found that the values of $K$ as obtained from eqn (2) were insensitive to variations in the ionic strength up to 0·26 mol dm$^{-3}$. Therefore, the values of $K$ obtained via eqn (2) were used in eqn (3). A plot of log $K$ versus $1/T$ is given in Fig. 5, which indicates that the values of $K$ as obtained in this study are in agreement with eqn (3). $\Delta H^\circ$ and $\Delta S^\circ$ were obtained from the slope and intercept of the corresponding line in Fig. 5, respectively. The values obtained are $\Delta H^\circ = -22{\cdot}3 \pm 0{\cdot}8$ kJ mol$^{-1}$ and $\Delta S^\circ = -20{\cdot}4 \pm 2{\cdot}7$ J mol$^{-1}$ K$^{-1}$ for the α-cyclodextrin inclusion process with the acid form of methyl orange. The corresponding values for the β-cyclodextrin inclusion process are $\Delta H^\circ = -10{\cdot}7 \pm 1{\cdot}0$ kJ mol$^{-1}$ and $\Delta S^\circ = 10{\cdot}9 \pm 3{\cdot}2$ J mol$^{-1}$ K$^{-1}$. Although the details of an inclusion process are not usually revealed by a thermodynamic study, some conclusions can be drawn from the above results. It is obvious that the inclusion process represented by eqn (1) is spontaneous and exothermic. The magnitude of $\Delta H^\circ$ for both α-cyclodextrin and β-cyclodextrin inclusion processes is indicative of weak intermolecular forces in the inclusion process. It is worth noting that $\Delta S^\circ$ is negative for the α-cyclodextrin complex, which means that methyl orange and α-cyclodextrin attain a more ordered state after the formation of an inclusion complex. Since several steps can be invisaged[4] for the inclusion process of a cyclodextrin, it is difficult to comment quantitatively on the values of $\Delta H^\circ$ and $\Delta S^\circ$ as reported in this study. The values of $\Delta G^\circ$ at 25°C are $-16{\cdot}2\,\mathrm{kJ\,mol^{-1}}$ for the α-cyclodextrin inclusion process and $-14{\cdot}0\,\mathrm{kJ\,mol^{-1}}$ for the β-cyclodextrin inclusion process and are in agreement with the values calculated from literature data[12] on $K$ at 25°C. Therefore, the acid form of methyl orange forms more stable inclusion complexes with α-cyclodextrin than with β-cyclodextrin. This result can be rationalized by considering the diameters of the cavities of both cyclodextrins. The α-cyclodextrin cavity has a small diameter compared with that of β-cyclodextrin. Therefore, there is a better fit for methyl orange in the cavity of α-cyclodextrin. The possibility of having the acid form of methyl orange binding to the cavity of γ-cyclodextrin was investigated in this study. It is found that γ-cyclodextrin does not induce measurable changes in the UV-visible spectrum of the acid form of methyl orange at temperatures down to 5°C. It is concluded that the acid form of methyl orange does not bind to the cavity of γ-cyclodextrin. It is interesting to note that γ-cyclodextrin was reported to bind the anion form of methyl orange in the dimer form.[13] The formation of dimer species of the acid form of methyl orange is unlikely under the experimental conditions used in this study.[14]

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